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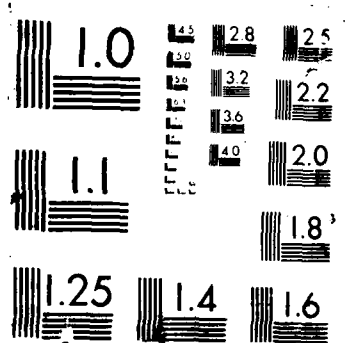
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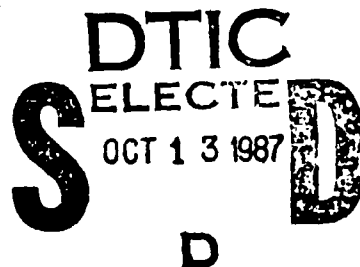
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REPORT

MRL-R-1064

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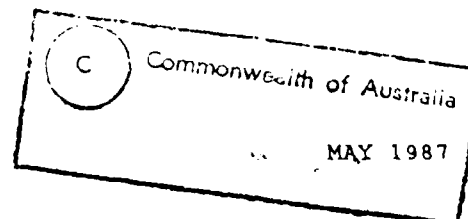
V.T. Borrett and A.G. Moritz



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ABSTRACT

Methods by which trace analysis using MS/MS techniques can be implemented on a VG7035 mass spectrometer are considered. Modifications which will permit fast scanning and multiple reaction ion monitoring are described. These techniques are suitable for direct mixture or GC-MS/MS analysis and are superior to linked scans using the Haddon method.



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TRACE ANALYSIS BY MS/MS ON A
DOUBLE FOCUSSED MASS SPECTROMETER

1. INTRODUCTION

Recent advances in mass spectrometry - mass spectrometry (MS/MS) indicate that this technique has a number of advantages over conventional methods for identification and trace analysis of organic materials [1]. The method involves detection of fragment or daughter ions which arise from spontaneous or collisionally activated decomposition of parent ions in a field free region of the spectrometer. -General features of MS/MS have been described in the literature by a number of authors [2-6]. The main advantages are the additional information provided in the form of generic relationships between ions in the mass spectrum and the high specificity which can be achieved. In many cases, data obtained by MS/MS is sufficient to differentiate between isomeric structures [7]. Although the ion intensity observed by MS/MS methods is considerably lower than with conventional MS, the reduction in "chemical noise" is generally sufficient to allow detection of compounds at low levels by direct mixture analysis [8,9].

In order to undertake MS/MS measurements, two or more analysers which can separate parent and daughter ions by either their mass to charge ratio, momentum or kinetic energy are required. For instruments which have a triple sector arrangement, the analysis and instrumental control circuits required to obtain constant daughter or parent ion spectra, or equivalent selected reaction ion monitoring are relatively straightforward. In these cases only one sector reference voltage needs to be scanned. Unfortunately, the high cost of triple sector instruments precludes their use in other than specialised laboratories. Although not specifically designed for the purpose, MS/MS measurements can also be undertaken on a conventional double focussed spectrometer. The major limitations are the low resolving power for the parent or daughter ions (depending on the geometry of the instrument) and the general requirement to link scan the reference voltages to control the magnetic field and electrostatic analyser.

It has been observed that spectra obtained under linked scanning conditions can be unreliable. In these circumstances linked scanning could be unsuitable for analyses in which a very high degree of certainty is

required. This report is a contribution to techniques for the verification of chemicals weapons usage, therefore it is essential that the reliability of such methods be assessed. This report covers some aspects of MS/MS as it applies to instruments of the 7070 type manufactured by Vacuum Generators, U.K., together with experimental approaches to improve the performance of MS/MS methods and to implement Multiple Reaction Ion Monitoring (MRIM). This work is part of Task AUS 85/051, sponsored by the Departments of Foreign Affairs and Defence.

2. EXPERIMENTAL

All measurements were made on a Vacuum Generators model 7035 double focussing mass spectrometer with conventional geometry. The instrument configuration includes a fully laminated magnet, model 3 digital scanner with dual (16 and 18 bit) digital to analogue converters, a PDP8 computer with 64K RAM, dual RL02 disk drives and a VT105 visual display unit.

The software (R8.0) [10] provides a linked scanning facility which operates in a similar way to that proposed and demonstrated by Haddon [11]. The method involves accurate calibration of the mass scale to allow simultaneous control of both the electrostatic analyser and magnetic field to maintain the B/E , B^2/E or $(1-E)^{0.5} B/E$ ratio constant. Data can be acquired either by peak detection or in a "continuum" mode with 3788 data points. The selective ion recording software allows for either voltage or magnetic switching but has no facilities for multiple reaction ion monitoring.

The instrument was modified to implement "Barber Elliot" type scans [12] with digital sweep of the accelerating voltage. The simplest method involves control of the accelerating voltage using the DAC normally used to control the magnet. This approach has the advantage of being able to use existing software for acquisition and control, as well as allowing the maximum accelerating voltage to be increased from 4 to 5 kV. For some experiments, independent digital control of the electrostatic analyser was achieved by means of a Varian 620L computer equipped with a 13 bit ADC and 10 bit DAC. For constant parent ion scans, the electrostatic analyser reference voltage was derived from the magnet reference voltage via two inverting operational amplifiers. The 620L computer provided corrections for non linearity of the Hall effect probe via a non-inverting input. With the Hall effect probe adjusted in the recommended manner, this method increases the effective resolution of the ESA reference voltage to 13 bits as well as minimising response time. Software for the computer correction of the Hall probe linearity involved a polynomial evaluation implemented in Horner form. Settling time for the control voltage was estimated to be less than 80 μ s.

3. RESULTS AND DISCUSSION

Experience with the VG 7035 instrument under main beam conditions has established that it is possible to maintain high stability for real-time mass measurements using either the Hites-Bieman method or accurate mass measurement algorithm [10]. The long-term reproducibility is such that calibration files can be maintained for periods of 6-9 months with deviations of less than 0.3 u. On the other hand, the stability for link-scanned measurements was relatively poor, particularly for constant daughter ion scans. In part, these differences arise from the problem of providing a lock mass (or secondary reference peaks) under linked scanning conditions. Based on an analysis of time and mass data under exponential scanning conditions, the major error can be attributed to changes in the start mass rather than to shifts in the scan law. This is to be expected for systems fitted with full digital sweep and field control.

An analysis of the linked scanning facility indicates that problems can arise in several areas of instrument operation. In the case of constant daughter ion scans, the method depends on implementation of the following steps,

1. minimising the offset in the electrostatic analyser (ESA)
2. decoupling the accelerating voltage reference voltage from the ESA
3. acquisition of data to enable an accurate and linked scan calibration file to be calculated
4. ensuring that the "start mass" is initiated within 100 ppm.

Calibration methods which use a previously derived accurate calibration file were found to be unsatisfactory as it is not possible to reset the accelerating voltage to the required accuracy. This implies that a new calibration file would have to be created immediately prior to the acquisition of linked scanned data. In general, the results obtained using the recommended procedures showed large variations in the relative intensity of the peaks between duplicate runs. In many cases, peaks which should be observed and can be established to exist by other methods, were completely absent in the linked scan spectrum.

Although some variations in peak intensities can be attributed to different life times of ions as a result of ion source tuning (particularly repeller voltage) the major limiting factor appears to arise from deviations which occur in the accurate calibration file. For daughter ions of low m/z ratio, e.g. 100 u, the combined error in the "start mass" and calibration should not exceed 50-100 ppm over the range $m/z = 28-100$ u. This value is approaching the resolution of the D/A converter at low magnetic fields and the short term stability of the system. Furthermore, the problem is exacerbated if minor reference peaks are not observed during calibration which leads to large interpolation errors. Scanning methods that obtain reproducible results for constant daughter ions or for constant neutral loss are experimentally much easier to obtain than those for constant parent ions. This is expected

because scans at constant B/E intersect the metastable peaks in the first field free region at an acute angle [4].

For the VG 7035, all linked scanning methods are limited to linear down scans at 3 s/decade which would restrict applications using combined gas chromatography to packed columns.

The operating requirements for all types of linked scans are considerably relaxed if both the entrance and exit slits are adjusted for near minimum resolution at the daughter ion mass and flat topped peaks. Measurements made using the continuum mode of data acquisition indicated only a minor reduction in resolution for constant daughter ion spectra. This observation is consistent with the theory that the peak widths in constant daughter ion spectra are primarily determined by kinetic energy release during fragmentation. Operation of the instrument at low resolution has the additional advantage of increased sensitivity. Measurements on various hydrocarbons and phosphonate esters indicated an improvement in sensitivity of a factor of ten.

Several methods were investigated to improve the acquisition speed in constant daughter or parent ion spectra. In one series of experiments, a 620L computer was used to control the ESA reference voltage (see experimental). The minimum scan rate that could be achieved was about 0.8 s/decade. Measurements indicated that a second degree polynomial was sufficient to reduce tracking errors to $\pm 0.2\%$ over one decade for scans at constant B/E. The results for hexadecane with a constant parent ion at $m/z = 226$ u were indistinguishable from those obtained using the Haddon method or those previously reported using scan speeds of 3 s/decade. Only minor further improvements could be achieved by an offset in the accelerating voltage which would compensate for settling time in either the magnet, 620L computer or the electrostatic analyser. Similar results were obtained by an alternative method in which the ESA voltage was derived directly from the magnet supply with a variable gain amplifier. Corrections for the non-linearity in the magnetic field control over a limited range (2:1) were implemented by an offset in the Hall probe circuits. Although the reasons for the limited scan speed are not understood, the method could be adopted for selective ion recording where appropriate channel delays can be used with existing software.

The problems which are inherent in linked scanning suggested that the method proposed by Barber and Elliot [12] be re-examined. The major advantage of this approach is that scanning only involves a single parameter (accelerating voltage) and that the mass scale is linear; that is, for the transition

$$m_1^+ \rightarrow m_2^+ + (m_1 - m_2) \quad (1)$$

the metastable ion appears at m^* where

$$m^* \approx m_2^2/m_1 \quad (2)$$

and the ratio of accelerating voltages (V_1 and V_2) required to transmit m_1 and m_2 respectively is given by

$$V_1/V_2 = m_1/m_2 \quad (3)$$

With digital sweep of the accelerating voltage and the slits adjusted for approximately 1000 ppm flat topped peaks, there are several other advantages, namely

1. Selective ion recording methods can be used without any modifications to software.
2. As the "precursor peaks" are relatively broad (~ 1 u at half height), the theoretical resolution and stability of the accelerating voltage is in excess of requirements by a factor of ~ 50 . In these circumstances, the overall stability is only limited by the stability of the field control under static conditions; it is not necessary to wait for the system to achieve equilibrium under cyclic conditions.
3. As only one parameter needs to be swept, there is no possibility that peaks will be lost or show distorted intensities, apart from defocussing effects where the accelerating voltage differs significantly from 4 kV, or where the overall gain of the detection system is inadequate.
4. With the existing accurate mass measurement software, the position of the metastable peaks can be obtained directly with an accuracy only limited by the detector signal to noise ratio (typically, ± 0.002 when using ρ, μ coordinates [6], where $\rho = EV_0/E_0V$ and $\mu = (E_0/E)m_B$).
5. As the 3 dB bandwidth of the programmed power supply is in excess of 500 Hz, there are no fundamental limitations in the scan speed apart from that which can be achieved by the digital scanner (0.05 s/decade). Measurements on hydrocarbon mixtures established that there was no difficulty in achieving sensitivity limits at the 100 picogram level at scan speeds of 0.2 s/decade under GC-MS/MS conditions. As might be expected, the sensitivity under MRIM conditions is increased by about an order of magnitude.

Operation of the instrument in the accelerating voltage scanning mode involves deriving the mass scale from time data using either centroid or peak top detection. This approach has several advantages over the recommended method involving linked scanning at constant B^2/E , namely automatic compensation for the tracking capability of the programmed power supply as well as compact data format (i.e. typically 6 peaks compared to 3788 data points for the continuum mode).

A convenient method of calibration is to use a normal alkane which provides a series of peaks spaced by 14 u. For example, using hexadecane with

a constant daughter ion at $m/z = 99$ u, peaks are observed corresponding to $m/z = 141, 155, 169, 183, 197$ and 226 u. A three point calibration (e.g. 99, 155 and 226 u) allows a cross check of the method and established that exponential scans can be accommodated by the calibration software. Conversion of the calibration file for any other daughter ion can be achieved by simple ratios either before data acquisition or by reprocessing of the time data.

In order to improve noise rejection, the minimum peak width (in terms of the number of sampled points) is best selected on test. For example a scan rate of 0.2 s/decade, typical peak widths are ~ 250 sample points for which a minimum peak width of 150 is satisfactory. Measurements on hexadecane indicate a reproducibility of ~ 0.3 u (Table 1). These results were obtained using a septum input and correspond to a 0.5 V (max. 10 V) signal at the ADC. Near the detection limit, the reproducibility is not sufficient to guarantee an accuracy within 1 u. This problem also occurs with linked scanning using the continuum mode. It should be noted that there are limitations using data acquisition with voltage scanning. For closely spaced peaks (e.g. separated by 2 u), weak peaks may be missed using peak detection or averaged in the centroid mode.

Calibration for Multiple Reaction Ion Monitoring is easily accomplished using a standard mass calibration file for magnetic SIR (Selective Ion Recording) and dummy masses to give the required ratio of V_1/V_2 . These values can be derived directly from the SETUP mode in FBSIR [10].

The two major limitations which are usually raised (13) with respect to accelerating voltage scanning are the limited mass range which can be covered and the distortion of relative peak heights. However, a limited mass range can also occur with linked scanning (constant daughter ion) using digital sweep. For example, a scan with a constant daughter ion at $m/z = 99$ u is limited to an upper mass of $99^2/28 = 350$. Although the instrument has not been tested over a wide range of compounds, only minor ($\sim 5\%$) variations in relative peak intensities have been observed where the accelerating voltage is varied over the range 3-5 kV (tuned at 4.0 kV, main beam signals at 2.0 kV). For an analytical application, such variations are not generally significant and in any case can be allowed for when an authentic sample is available for direct comparison.

The problem of distortion of peak heights in linked scan spectra does not appear to be adequately covered in the literature and raises the question of the best experimental method by which such data can be acquired. One approach, which eliminates problems of accurate alignment of the B and E fields is to use localised B (or E) scans with a constant accelerating voltage decoupled from the electrostatic analyser. Although not described in the VG software handbooks, this method can be implemented using the "Acquisition" set up mode. Details of the method are given in Appendix A.

4. CONCLUSIONS

The standard facilities provided for linked scanning on the VG 7035 mass spectrometer are unsuitable for low or trace level analysis by MS/MS. The principal limitations are the relatively slow scanning speed (3 s/decade), poor stability (particularly for constant daughter ion scans) and absence of procedures for multiple reaction ion monitoring (MRIM). Improvements in the stability and sensitivity can be achieved by non-standard procedures, namely by operating at the lowest possible resolution and with flat-topped peaks.

Fast scanning (0.1 s/decade) and MRIM with high stability and sensitivity are possible by modifying the instrument for scanning of the accelerating voltage or by independent computer control of the electrostatic analyser. Both methods are suitable for acquisition of the data by the existing computer system.

Direct analysis of mixtures at trace levels can be implemented on the VG 7035 instrument using existing foreground - background selective ion recording software. The method is suitable for chart recording over a wide dynamic range for any combination of main beam or selective reaction ion monitoring at constant accelerating voltage.

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TABLE 1

Statistical analysis for hexadecane, constant daughter-ion spectrum
at $m/z = 99$ u. $V_0 = 2$ kV, scan rate 0.2 s/decade, centroid data.

| MASS | INTENSITY* | σ † |
|------|------------|------------|
| 226 | 38 | 0.13 |
| 197 | 37 | 0.13 |
| 183 | 42 | 0.18 |
| 169 | 87 | 0.18 |
| 155 | 100 | 0.17 |
| 141 | 75 | 0.13 |

* Average of 100 measurements, relative to $m/z = 155$ u.

† Average standard deviation in mass (σ_m) = 0.33

APPENDIX A

MULTIPLE REACTION ION MONITORING WITH

THE 7070 SYSTEM

Any combination of transitions in the 1st or 2nd field free regions, or main beam signals can be monitored on 7070 systems equipped with a digital scanner (dual DAC's) and FBSIR software. The method takes advantage of the fact that after "CTRL/L" in "Acquisition" setup mode, the gain and control voltages for both the ESA and magnet on any channel are freely programmable. These parameters are stored and can be used in setup procedures with the "H" command. No modifications to hardware or software are required. The method has been found useful for probe or septum samples and for checking results obtained by linked scanning. The operating procedure is outlined below.

1. In setup mode, focus any strong ion in normal coupled mode ($A = 980,000$), decouple PPS2 and refocus with manual control.
2. Enter "Acquisition" setup mode with any method file which has the required number of channels. After "CTRL/L" insert required values of ESA and magnet control voltages i.e. ESA voltage set for 980,000 for main beam signals and transitions in the second field free region (FFR) and reduced by the ratio m_2/m_1 for transitions in the first FFR. Magnet control voltages can be determined directly from a magnet calibration file with values set for m_1 , m_2 or $m^* = m_2^2/m_1$ in the case of metastable transitions.
3. Hard copy requires a chart recorder to be connected after the programmable amplifier in the digital scanner. A suitable (1V) high impedance floating output can be taken across resistor R6 (10K) located on board 547 in the 2200 interface. Glitches which arise from the absence of SIR delay can be minimised by adjusting the span width to exceed that of a metastable transition.
4. Optimum sensitivity for metastable transitions is obtained with the system operated at the lowest possible resolution (depends on the highest mass involved) and flat-topped peaks.

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